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Pergamon

Life Sciences, Vol. 56, No. 9, pp. 637-660, 1995

0024-3205/95 $29.00 + .00

0024-3205(94)00427-7

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MINIREVIEW

METABOLISM AND ACTIONS OF CDP-CHOLINE AS AN ENDOGENOUS COMPOUND AND ADMINISTERED EXOGENOUSLY AS CITICOLINE

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(Received in final form November 8, 1994)

Summary

CDP-choline, supplied exogenously as citicoline, has beneficial physiological actions on cellular function that have been extensively studied and characterized in numerous model systems. As the product of the rate-limiting step in the synthesis of phosphatidylcholine from choline, CDP-choline and its hydrolysis products (cytidine and choline) play important roles in generation of phospholipids involved in membrane formation and repair. They also contribute to such critical metabolic functions as formation of nucleic acids, proteins, and acetylcholine. Orally-administered citicoline is hydrolyzed in the intestine, absorbed rapidly as choline and cytidine, resynthesized in liver and other tissues, and subsequently mobilized in CDP-choline synthetic pathways. Citicoline is efficiently utilized in brain cells for membrane lipid synthesis where it not only increases phospholipid synthesis but also inhibits phospholipid degradation. Exogenously administered citicoline prevents, reduces, or reverses effects of ischemia and/or hypoxia in most animal and cellular models studied, and acts in head trauma models to decrease and limit nerve cell membrane damage, restore intracellular regulatory enzyme sensitivity and function, and limit edema. Thus, considerable accumulated evidence supports use of citicoline to enhance membrane maintenance, membrane repair, and neuronal function in conditions such as ischemic and traumatic injuries. Beneficial effects of exogenous citicoline also have been postulated and/or reported in experimental models for dyskinesia, Parkinson's disease, cardiovascular disease, aging, Alzheimer's disease, learning and memory, and cholinergic stimulation.

Key Words: CDP-choline, neuronal function, metabolism, cellular membranes, citicoline

Cytidine-5'-diphosphate choline (CDP-choline; citicoline), a naturally occurring endogenous nucleoside, is an intermediate compound in the major pathway for the biosynthesis of phos-

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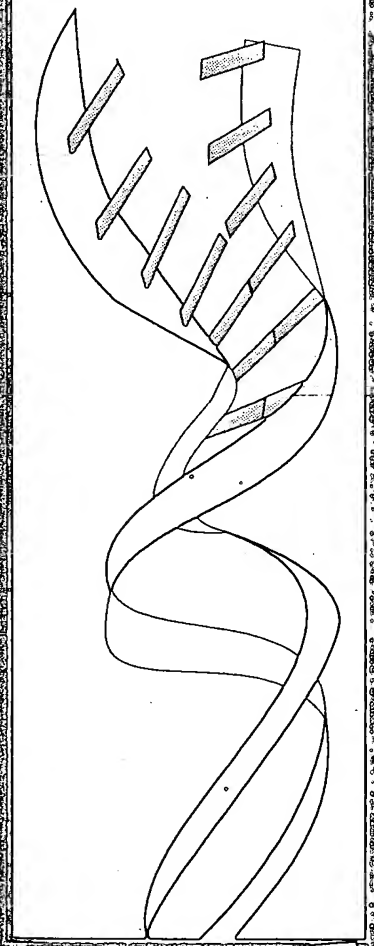

MOLECULAR AND
CELLULAR ASPECTS OF:

PHARMACOLOGICAL AND AUTONOMIC
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0024-3205(1995)56:9;1-C

phatidylcholine. This pathway, frequently termed the Kennedy pathway, has been thoroughly characterized in numerous studies since it was initially described (1, 2). Current knowledge about the enzymology and regulation of phosphatidylcholine biosynthesis has been summarized in a recent review (3). Phosphatidylcholine has been of primary research interest as the major and essential phospholipid component of cellular membranes as well as in lipoprotein synthetic pathways. CDP-choline has also received increased attention over the preceding two decades as it became clear that the synthesis of this compound represented the limiting step in the Kennedy pathway.

CDP-choline ($C_{14}H_{26}N_4O_{11}P_2$) is a polarized molecule with a molecular weight of 488.33 (Fig. 1). The monosodium salt of CDP-choline (MW=510.31) is a white, crystalline, spongy, very hygroscopic powder that is soluble in water and almost insoluble in alcohol (4). Citicoline is the proposed International Non-proprietary Name (pINN) for cytidine-5'-diphosphate choline. The compound has been studied for many years in numerous countries throughout the world under more than 50 trade names. The concept that administration of exogenous CDP-choline (citicoline) might augment endogenous phosphatidylcholine synthesis is attractive because accelerated replacement or repair of cellular membranes could play a critical role in the function of numerous physiological processes. It could have potential therapeutic efficacy in a variety of diseases in which membrane disorder, dysfunction, or degeneration results in cellular and tissue ischemia and necrosis.

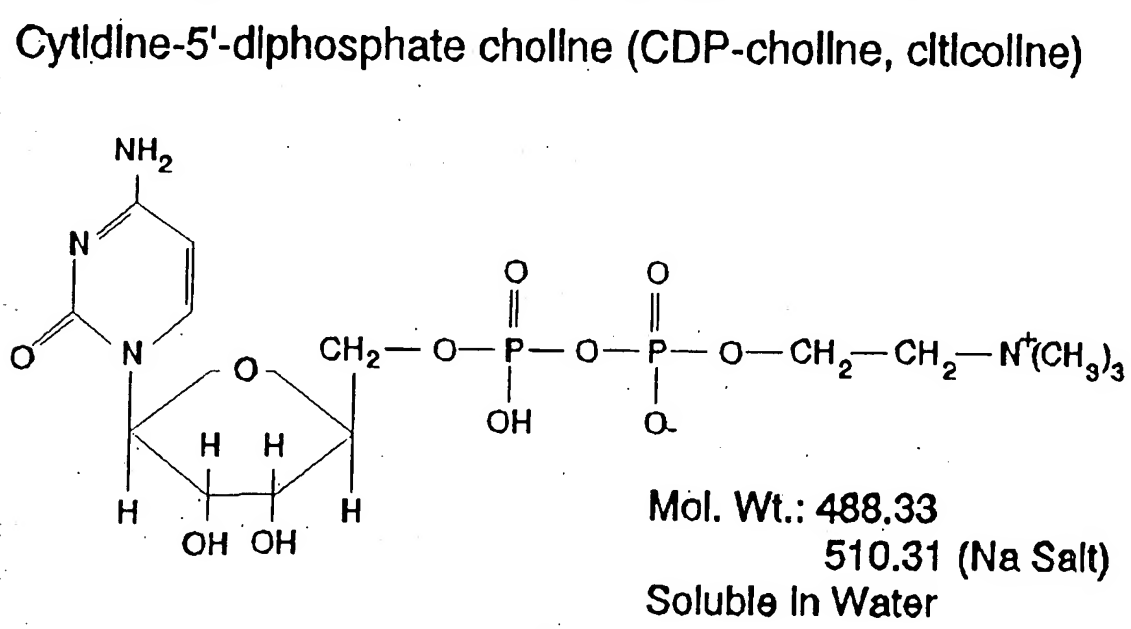

Fig. 1

Chemical structure of cytidine-5'-diphosphate choline (CDP- choline, citicoline).

Metabolism

CDP-choline is synthesized from cytidine-5'-triphosphate and phosphocholine with accompanying production of inorganic pyrophosphate in a reversible reaction (5) catalyzed by the enzyme CTP: hosphocholine cytidylyltransferase (6). CDP-choline can be readily hydrolyzed in vivo (Fig. 2) to cytidine monophosphate and phosphocholine by phosphodiesterases in the cell wall, and subsequent dephosphorylation of both molecules was postulated to then precede uptake into the cell and intracellular enzymatic resynthesis of CDP-choline (7). In the neuronally-related PC12 cells (8), it

was shown that exogenous ^{3}H-cytidine was readily incorporated into the cells and mainly converted into cytidine triphosphate, and also that cytidine increased ^{14}C-choline incorporation into membrane phosphatidylcholine. In a similar manner, rates of CDP-choline and phosphatidylcholine synthesis were stimulated 4.5-fold in rats fed orotic acid, a cytidine precursor (9).

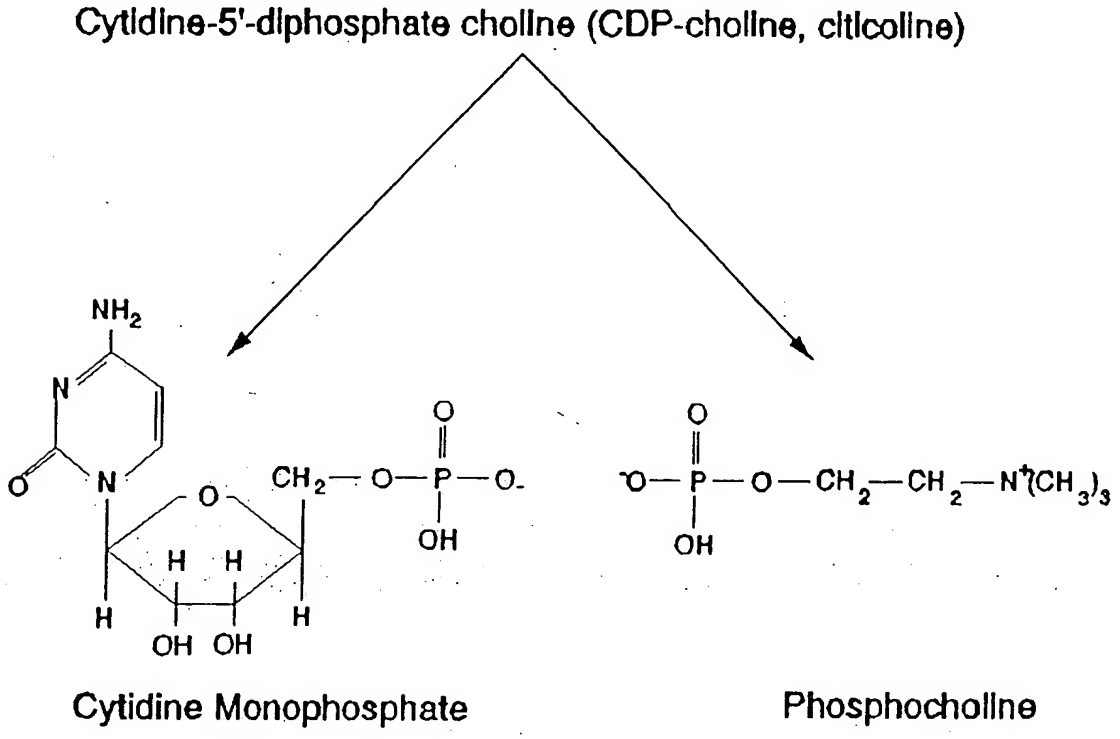

Fig. 2

Chemical structure of hydrolysis products of cytidine-5'-diphosphate choline (CDP-choline, citicoline).

The three-step synthetic pathway (Kennedy pathway) leading from choline to phosphatidylcholine was initially reported in 1956 by Kennedy and Weiss (2), and was reviewed recently in considerable detail (3). In the initial step, the phosphorylation of choline to phosphocholine is catalyzed by the enzyme choline kinase. The second and rate-limiting step is the previously mentioned formation of CDP-choline from cytidine-5'-triphosphate and phosphocholine. The rate-limiting nature of this step has been demonstrated in numerous preparations including chick brain microsomes (10), cultured chick embryonic muscle (11), cultured chick heart cells (12), rat hepatocytes (13, 14), rat myoblast (L6) cell line (15), rat lung type II cells (16), hamster heart (17), and guinea pig heart (18). The final step is the reaction (catalyzed by the enzyme CDP-choline:1,2-diacylglycerol cholinephosphotransferase) of CDP-choline with diacylglyceride to form phosphatidylcholine and monoglyceride.

The Kennedy pathway is not the only biosynthetic route to phosphatidylcholine (19, 20), but it appears to be the dominant one in many types of cells. For example, over 90% of cardiac phosphatidylcholine is synthesized via this pathway (21). The pathway has been identified and characterized in numerous cells and tissues, including primary cell cultures from rat brain (22), rat lung microsomes (23), rat lung type II cells (24), hepatocytes (25, 26), rat renal cortex (27, 28), fetal rat lung (29), fetal

rabbit lung (30), rat brain (31-33), rat liver (34), rat skeletal muscle (35), isolated hamster heart (17), isolated perfused guinea pig heart (18), and isolated perfused rabbit lung (36).

Within specific tissues and cells, there appears to be localization of pathway enzymes. Differing rates presumably corresponding to differing metabolic activities have been reported in rabbit neuronal and glial cells (37), and activity appears to be localized in the microsomal fraction from yeast cells (38) and rat brain white and gray matter (39). Evidence has accumulated that synthetic activity is concentrated in the external or outer side (corresponding to the cytoplasmic side in vivo) in chicken and rat brain microsomes (40) and rat liver microsomes (41, 42). Synthetic activity has also been reported in rat brain synaptic plasma membrane (43, 44) and in local areas of rat sympathetic neurons (45).

In addition to anatomical localization of the Kennedy pathway activity, there is considerable experimental evidence that, at different steps in the pathway, different pools contribute variably to the final phospholipid product. In rat hepatocytes, radiolabeled precursors for the Kennedy pathway or alternate pathways yield differing ratios of labeled phospholipid at different anatomical locations (46). Even within the pathway, when choline and cytidine were labeled with different isotopes (^{3}H or ^{14}C) in cultured brain cells (7) or when citicoline and diacylglycerol were synthetically labeled with different isotopes in rat lung microsomes (47) or with the same isotope (^{14}C) in parallel experiments in rat brain microsomes (48), the rates at which the labels were incorporated in membranes or cells differed. These differing rates of synthesis for the two labeled components were interpreted as indicating that endogenous nonuniformly mixing substrate pools exist. Spatially separate pools may, in turn, be shown to be specifically regulated as in the cases of structurally localized inhibition by hemicholinium-3 of choline transport in cultured glioma cells (49), synthetic rates dependent upon intracellular calcium concentrations in cultured glioma cells (50) or rat hepatocytes (51), or dependent upon amino acid concentrations in rat cortical cells (52), and regulation of the phosphocholine pool size by phosphocholine phosphatase in the hamster heart (53).

The metabolic pathways of particular importance for CDP-choline and its hydrolysis products are outlined in Figure 3. The hydrolysis and dephosphorylation of CDP-choline as well as the Kennedy synthetic pathway to phosphatidylcholine were discussed earlier in this section. Choline is also the precursor for two other major pathways, synthesis of the neurotransmitter acetylcholine (54) and resynthesis of methionine via betaine aldehyde and betaine with subsequent transmethylation to homocysteine and to methionine (55). However, the latter synthesis (betaine-homocysteine transmethylation) does not occur in brain cells, which cannot utilize choline or betaine for this synthesis and must obtain the methylated compounds from external sources (56). The pyrimidine, cytidine, is widely distributed and incorporated into cellular nucleic acids. In addition to these primary pathways, there are a number of other relevant physiological reactions. For example, CDP-choline is a critical precursor for the synthesis of platelet-activating factor (57-59) and also has been reported to affect the activity of cytochrome oxidase and other enzymes involved in energy transduction in rat brain homogenates (60, 61). Additionally, phosphatidylcholine synthesis is increased by numerous agents including thyrotropin-releasing hormone and phorbol esters in GH_3 pituitary cells (62), glucose in rat pancreatic islets (63), and gemfibrozil in cultured rat hepatocytes (64), whereas α-adrenergic stimulation decreases phosphatidylcholine synthesis (65).

Thus, the diverse products of CDP-choline and its metabolites can affect a number of biologically critical parameters and can be modulated by diverse agents. Documentation of the synthesis and metabolism of CDP-choline can provide a basis for identifying those actions providing the primary underlying mechanism(s) for pharmacological activity initiated by administration of citicoline.

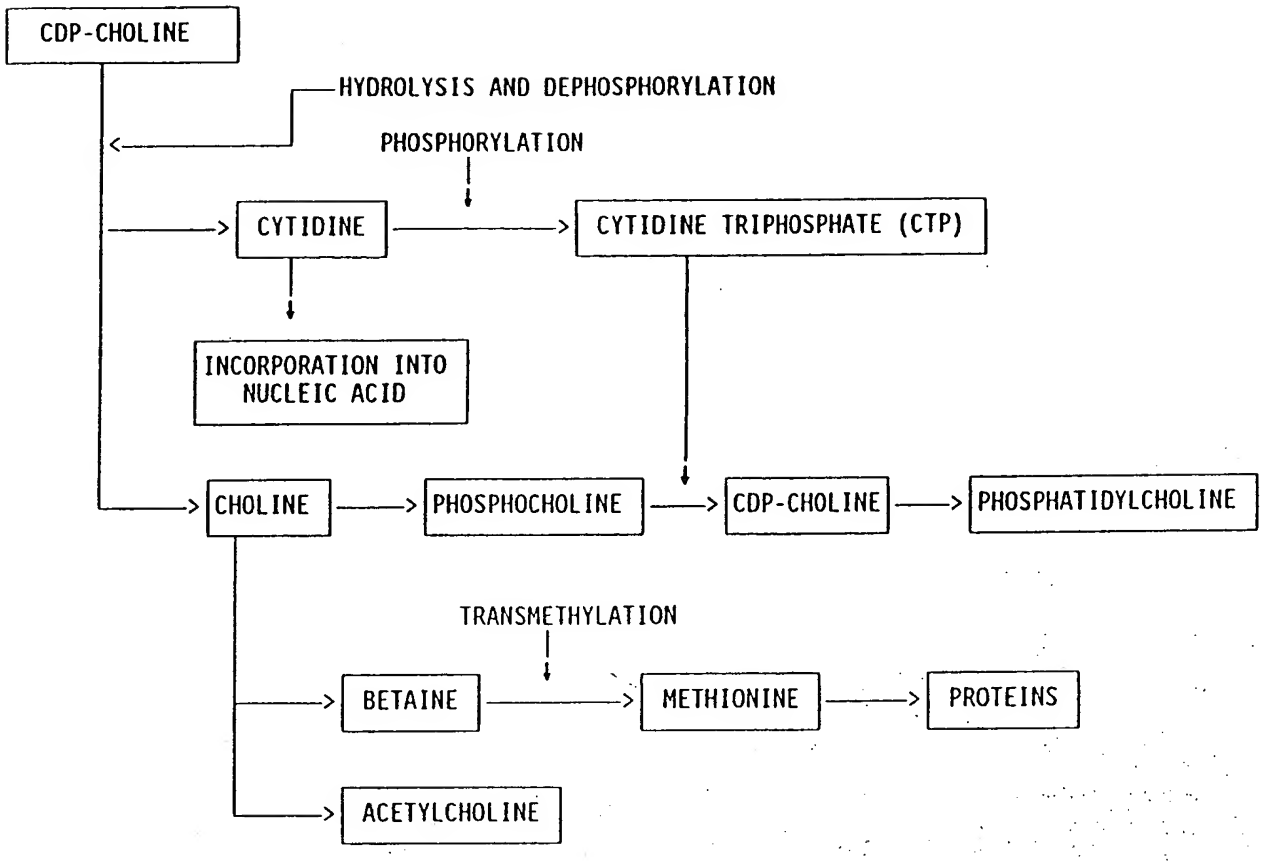

Fig. 3

Summary of major metabolic pathways for CDP-choline.

Pharmacokinetics

The synthesis of citicoline with ^{14}C at the methyl carbons of the choline moiety and ^{3}H in position 5 of the cytidine nucleus has been described (66). Use of this product with each of the two dissociable components labeled differently yields particularly useful information because analysis of cytidine and choline pharmacokinetics in the same experiment can be performed. More limited information is obtained in studies with only one part of the molecule labeled, with both parts labeled with ^{14}C in the same experiments, or with each of the two components labeled with ^{14}C in parallel experiments.

The oral absorption and subsequent initial distribution of citicoline has been measured with radioisotopically-labeled citicoline in several different studies. In a series of experiments with citicoline labeled with either ^{14}C or ^{3}H in the positions described above, the intestinal absorption patterns of citicoline and/or its metabolites were delineated (67). Thirty minutes after oral administration to rats of either labeled compound, analysis of stomach and intestinal contents indicated that the bulk of radioactivity in the stomach was in the citicoline fraction, whereas the radioactivity present in the intestine was predominantly in either the choline fraction or the cytidine fraction. It was postulated that elevated relative amounts of phosphorylated choline and cytidine in the intestinal mucosa compared to the lumen indicates active transport across the intestinal mucosa in these forms. The bioavailability of methyl-^{14}C citicoline in rats was almost complete with estimates of 92% (68)

and 94.5% (69) in two different studies. Measurements with the double-labeled citicoline molecule document that differential uptake and utilization of cytidine and choline portions of the molecule from the intestine occur with most isotope being absorbed within a few hours (67, 70). Comparison of radioactivity distribution after oral and intravenous administration of citicoline shows some quantitative differences, primarily a slower rise in vascular concentrations and a higher urinary excretion (presumably of gastrointestinal metabolites) with the oral route, after a 300 mg/kg dose (69), as well as approximately twice as much choline component uptake into the liver (67). In rats, after a single bolus injection (50 mg/kg citicoline) plasma citicoline levels detectable after 1 minute (2.9 μM) fall to undetectable levels 5 minutes later, and plasma cytidine levels also increase rapidly (from 1.5 to 124 μM) and remain elevated for at least 60 minutes (71). The speed of the intestinal citicoline hydrolysis appears to be the limiting factor in the absorption process (72). After entering the circulation, most of the isotope is rapidly distributed to body tissues with the highest concentration in the liver (67, 70).

In experiments with double-labeled isotope, preferential uptake of the ^{14}C (choline) portion occurred in the liver (67, 70), with the ^{3}H (cytidine) portion more widely distributed and apparently incorporated into the nucleic acid fraction via the cytidine nucleotide pool (70). The observed differential distribution of the two portions of citicoline was interpreted as supporting the concept that choline and cytidine are absorbed and taken up into cells separately. Pharmacokinetics of orally administered citicoline were measured in liver and brain in both rats and dogs, and no significant differences were observed between these two species (70). In liver, the ^{14}C-labeled choline and choline metabolite levels increase rapidly and then rapidly decrease (after the first hour) as metabolites derived from choline are released into the circulation with the resulting peak plasma level attained after 4 hours (72). The percentages of ^{14}C and ^{3}H metabolites present in rat liver were measured (56, 70, 72, 73). High initial levels of cytidine, cytidine nucleotides, betaine and phosphocholine are replaced at the longer time interval by increased levels of nucleic acids, methionine, proteins and phospholipids. The uptake of radioactivity derived from citicoline was studied in perfused rat liver (74). Within 5 minutes, the double-labeled citicoline was largely degraded to ^{3}H-cytidine monophosphate and ^{14}C-choline. Thus, uptake by perfused liver cells, at least after a short initial interval, can be associated with metabolites of citicoline. From the accumulated metabolites, citicoline and phosphatidylcholine can be readily resynthesized via the Kennedy pathway. Experiments with isolated rat liver microsomes and mitochondria (75) demonstrate that phosphatidylcholine can be synthesized from citicoline in both fractions, especially mitochondria. Much of the isotope accumulated in the two major uptake sites (liver and kidneys) as well as in other organs appears to be incorporated into phospholipids, nucleosides, and proteins, and retained by the cells with only minor amounts excreted by the gastrointestinal tract, kidneys, or (as CO_2) lungs (67, 68, 69, 72).

Uptake of radioactivity into brain after either oral or intravenous administration of citicoline differs from that observed in any other tissues. Only a very small amount of the total citicoline administered is taken up as choline and cytidine by the brain, but the utilization of those quantities taken up for the synthesis of endogenous CDP-choline and, subsequently, for phospholipid and other biosynthesis is highly efficient (70). The ^{3}H-cytidine moiety is more rapidly accumulated with the ^{14}C incorporation slowly increasing for up to 5 hours (70), and no decreases in radioactivity levels are observed for up to 48 hours (56, 67). Though only 0.25% of the radioactivity in the administered oral dose was present in the rat brain homogenate, 62.8% of this was in the lipid extract after 24 hours, mainly as phosphatidylcholine (76.5%) and sphingomyelin (17.9%) (76). The percentages of ^{14}C and ^{3}H metabolites present in rat brain were measured (56, 70, 73). High initial levels of cytidine, cytidine nucleotides, methionine, and phosphocholine are replaced at the longer time interval by increased levels of nucleic acids, proteins, and phospholipids (70). Autoradiographic measurements

of ^{14}C-labeled citicoline after either intravenous or intrathecal administration in rats (77, 78) indicated that, in specimens prepared 1 hour after infusion, the compound was detectable in the brain and spinal cord only after intrathecal injection (with more immediate access to the brain sites).

Several radioautographic studies were performed with ^{14}C-methyl labeled citicoline to examine subcellular distribution in brain. Low-resolution autoradiography in rat brain detected labeled material in the neurons 24 hours after administration, indicating intracellular incorporation (79). Similar high-resolution autoradiography in mouse brain 24 hours (80) or 10 days (81) after administration indicated increased radioactivity in cellular membranes without further selectivity (80) and in cholinergic neuronal areas in brain and subcellular membranes in Purkinje cells in the cerebellum (81). In a similar but nonradioautographic study in rats (82), brains were homogenized and fractionated 24 hours after compound administration, and radioactivity was found to be associated with cytoplasmic and microsomal membrane fractions. Furthermore, in cultured fetal rat brain cells incubated with double-labeled citicoline (83), different intracellular ^{3}H/^{14}C ratios from those in the extracellular incubation solution were identified, and a rapid subsequent labeling of phosphatidylcholine was detected. Thus, as in other cell types, choline and cytidine are taken up separately and mix with similar cellular pools, and then citicoline is resynthesized and used for cellular phosphatidylcholine synthesis. In cultured cells from chick ganglia (84), radioautography of ^{3}H-labeled citicoline indicates that the citicoline was taken up into nerve cells and initially incorporated primarily into the nucleus within 2 hours. In similar experiments (84), glial (kitten cerebellar astraglia) cells and ependymal (human ependymoma) cells did not incorporate as much citicoline as did nerve cells.

Pharmacodynamics

Ischemia/Hypoxia. Biosynthesis of membrane phospholipids is, clearly, of primary importance in dynamic regulation of cellular integrity. The role of phospholipids in maintenance of neuronal function in the highly lipid central nervous system has been of particular interest in circumstances where brain function has been compromised. In particular, considerable preclinical research and clinical interest have focused on measurement of phospholipid deficiencies in a variety of ischemic and hypoxic animal models as well as of the effects of exogenous citicoline administration in limiting deleterious changes observed in these experimental models. The application of these results and of those obtained in head trauma models to corresponding clinical situations appears to provide the most logical rationale for clinical potential and utility for citicoline. Various actions of citicoline reported in 10 different ischemia/hypoxia models in six species are summarized in Table I and discussed in this section.

Several studies were performed with a global model of ischemia in which ischemia was induced by incubating decapitated rat heads for 5 minutes at 37°C (85-88). In this model, intracerebral injection of CDP-ethanolamine and citicoline (0.6 μmoles of each) prevented ischemia-induced loss of radioactivity from choline and ethanolamine glycerophospholipids and from increased free fatty acids (85, 87, 88). The ischemia-induced decline in Na^+,K^+-ATPase activity, similar to that produced by addition of exogenous diacylglycerols to the ATPase assay mixture, was not prevented by pretreatment with citicoline and CDP-ethanolamine (86). Since accumulation of free fatty acids was affected by this pretreatment, it was concluded that local accumulation of diacylglycerols following ischemia is responsible for inhibition of ATPase.

The effects of citicoline on neurologic deficits and cerebral glucose metabolism were studied in a rat model in which transient cerebral ischemia was induced by occluding both common carotid arteries for 20 or 30 minutes, 24 hours after the vertebral arteries were permanently occluded by electrocautery (89). After reestablishing carotid blood flow, citicoline was given intraperitoneally at doses of 50 and 250 mg/kg twice daily for 4 days. The higher dose of citicoline in ischemic rats improved neurologic signs and attenuated increases in glucose and pyruvate levels and decreases in the synthesis of labeled acetylcholine from labeled glucose. It was postulated that citicoline may decrease the effects of cerebral ischemia by restoring glucose metabolism through a stimulant effect on phospholipid biosynthesis. In a similar model after 10 minutes of occlusion (90), groups of rats received different drugs including citicoline (76.1 and 152 mg/kg, intravenously) and effects on survival of hippocampal CA1 neurons (neuronal density) were subsequently measured. S-adenosyl-L-methionine did protect, but no protective effect was observed with citicoline or other compounds tested under similar conditions.

In an ischemic and anoxic model (91), the rat right carotid artery was ligated and rats were exposed to nitrogen for approximately 4 hours (until convulsions occurred). Test drugs were administered 20, 80, and 140 minutes after the anoxic treatment, and the effects on neurological deficits were assessed 48 hours after the anoxia treatment. Citicoline (300 mg/kg, intraperitoneally) significantly decreased the incidence of neurological deficits.

In several studies (92-95), the effects of acute hypobaric hypoxia were tested in rats subjected to differing atmospheric pressures in a hypobaric chamber. In the earliest study (92), citicoline (20 mg/kg) was given intraperitonally 18 hours before various parameters were measured, mainly during short-term exposure to lowered pressures of 500 mm Hg and 300 mm Hg (equivalent to 7180 m altitude). There was a reduction in vegetative (including hemodynamic) responses with no modifications of cerebral blood flow, preservation of the conditioned avoidance response, and stabilization (decreased reduction) of cerebral levels of dopamine and norepinephrine. In subsequent studies, effects of citicoline on changes in catecholamine levels elicited with hypobaric hypoxia were delineated. In rats exposed to hypobaric pressure of 300 mm Hg for 60 minutes before sacrifice, dopamine uptake was increased (95). When citicoline was given orally (1 g/kg for 3 days), synaptosomal uptake of dopamine was decreased. At similar hypobaric pressures for 15 minutes (93), citicoline (300 mg/kg orally per day for 7 days) protected from the effects of hypobaric hypoxia in behavior/conditioning tests. Further experiments were performed to determine whether citicoline (1 g/kg orally for 1 or 3 days) opposed the effects of hypobaric hypoxia (300 mm Hg maintained for 60 minutes) on catecholamine metabolism in rat striatum and hypothalamus (94). Hypoxia decreased striatal norepinephrine and several metabolites and increased both striatal and hypothalamic dopamine; citicoline partially reversed the changes in norepinephrine and dopamine levels and had differing effects on metabolite levels. Thus, it appears that citicoline inhibited the impairment by hypoxia of neurotransmitter release patterns.

A chronic hypoxia rat model was developed (96-98) by placing the animals in chambers in which the oxygen content was reduced stepwise (15, 12, 10, 8, and 7% O_2) over extended time periods, and their behavioral reactions indicative of deterioration in vigilance were recorded. In the initial study (96), citicoline (100 mg/kg in food) had a protective effect by increasing vigilance under mild (15% O_2) hypoxia. In a second study (97), rats were kept at chronic hypoxic levels of 15, 12, 10, and 7 vol% O_2 for 5 months, and their behavior in an open field was observed. Hypoxia-induced behavioral deteriorations were partially reduced by citicoline (100 mg/kg in food), and survival time was increased at 7 vol% O_2. The effects of a similar dose of citicoline on survival time in extreme (7 vol% O_2) hypoxia were confirmed in two experiments over a period of 6 months (98).

TABLE I

Effects of Citicoline on Ischemia and/or Hypoxia in Preclinical Models

Model	Species	Effects of Citicoline	Reference(s)
Decapitation ischemic model	Rat	Prevents free fatty acid release and glycerophospholipid degradation (but not decreased Na^+-K^+-ATPase activity)	(85–88)
Transient cerebral ischemia	Rat	Shortened time for the return of spontaneous motor activity; improved neurologic signs; decreased changes in glucose, pyruvate and acetylcholine	(89)
Transient cerebral ischemia	Rat	No effect (only S-adenosyl-L-methionine protects against neuronal death	(90)
Ligation and anoxia	Rat	Decreased incidence of neurological deficits	(91)
Acute hypobaric hypoxia	Rat	Reduced vegetative response parameters; protected conditioned avoidance response; partially restored learning performance; stabilized dopamine (decreases) and norepinephrine (increases levels	(92–95)
Chronic hypoxic hypoxia	Rat	Reversed long-term behavioral changes and mental deterioration; increased survival time	(96–98)
Ischemia model	Gerbil	Prevents increased fatty acids (in some conditions) and neutral lipids as well as decreased phosphatidylcholine	(99–101)
Hypoxia	Guinea pig	Reversed effect on RNA and protein labeling; increased phospholipids, especially in mitochondria	(102, 103)
Cerebral microvessel injury	Rabbit	Delayed vasodilating activity	(104)
Acute hypoxia	Dog (Beagle)	Increased synaptosomal phosphorylation only after hypoxia	(105)
Embryonic neuronal cultures	Chicken	Protected neurons from hypocapnic injury; increased cellular proliferation	(106)

In the gerbil model (99-101), brain ischemia is produced by bilateral ligation of the carotid arteries. In one study (101), citicoline (0.6 μmol/brain) was injected intraventricularly 5 minutes before ligation. Changes in brain free fatty acids, neutral lipids and different phospholipids, which were all labeled by intraventricular injection of ^{14}C-arachidonic acid 2 hours before ligation, were measured after 10 minutes of ligation-produced ischemia. Citicoline partially prevented the ischemia-induced increases in fatty acids and neutral lipids and the decrease in brain phosphatidylcholine. In a similar study (99), citicoline prevented approximately half of the ischemia-induced increase in the free fatty acid pool and in labeled fatty acid release as well as both the increase in diacylglycerol and the major portion of the decrease in phosphatidylcholine. In another series of experiments (100), which were similar except that citicoline (9 mg; approximately 150 mg/kg) was administered intraperitoneally 1-2 hours before bilateral ligation, the effects observed were decreased. The ischemia-induced increase in diglycerides was prevented, and the decrease in phosphatidylcholine was partially inhibited.

The pool size of endogenous citicoline (CDP-choline) was measured by radioactive isotopic (^{14}C) dilution in brains, hearts, and lungs from normal and hypoxic guinea pigs (102). Hypoxia was induced by intermittent (17 hours/day for 4 days) exposure to 9% oxygen in nitrogen, and citicoline (100 mg/kg, intraperitoneally) was administered 15 minutes before sacrifice. CDP-choline was decreased significantly in brains and lungs of hypoxic guinea pigs, and citicoline had no effect on pool sizes of CDP-choline in normal or hypoxic animals (concentrations of precursors or products were not measured). The effects of citicoline on hypoxia-induced decreases in incorporation of the labeled precursors, ^{3}H-glycerol and ^{14}C-palmitate, into total lipids and into phospholipids of mitochondria isolated from cerebral hemispheres, cerebellum, and brainstem were measured (102). Citicoline increased total lipid and phospholipid levels in mitochondria purified from all three brain regions of hypoxic guinea pigs, indicating that citicoline restored lipid metabolism most in those membranes most affected by the hypoxic treatment. In an additional study employing similar approaches (103), citicoline was not able to reverse the inhibitory effect of hypoxia on DNA labeling, but was able to remove the effect of hypoxia on RNA and protein labeling. The labeling of RNA in cerebellar nuclei and mitochondria and of proteins in microsomes from all three brain regions examined was particularly sensitive to citicoline, indicating a stimulation or restoration of energy metabolism.

A study was performed comparing the effects of SK-827 with those of citicoline (100 mg/kg, intravenously) and nicardipine on rabbit cerebral microcirculation with cerebral microvessel injury induced by glass bead injection into an internal carotid artery (104). Citicoline tended to dilate small arteries and larger arterioles after 10 minutes following the beginning of a 30-minute infusion, with a significant increase in cerebral blood flow observed.

Synaptosomal fractions obtained from the motor area of the cerebral cortex of normocapnic, normoxic, or hypoxic untreated beagle dogs or similar dogs treated with citicoline infusion were incubated and analyzed for ATP, ADP, AMP, creatine phosphate, pyruvate, and lactate (105). Perfusion with citicoline increased synaptosomal phosphorylation after hypoxia but had no effect after normoxia, indicating a specific protective action in hypoxia.

Neuronal cultures from chick embryo cerebral hemispheres were protected against a hypocapnic injury by adding 1 μM citicoline to their growth medium before or after the injury (106). Morphometric analysis showed that pretreatment of neuronal cultures with citicoline maintained the number of cell aggregates and of primary neuronal processes at control values after neuronal shock. Incubation of neurons with radioactive choline demonstrated that hypocapnia increased label

incorporation into phospholipids, whereas the presence of citicoline reduced it. Thus, citicoline appears to have the capacity to protect neurons and to maintain cellular function and growth.

Though the role of CDP-choline in the Kennedy pathway clearly indicates how this intermediate increases synthesis of phosphatidylcholine, the observed decrease elicited by administered citicoline in ischemia-induced free fatty acid release and in diglycerides indicates that citicoline can also prevent the loss of membrane phosphatidylcholine. The mechanism by which this could occur has been discussed in some detail (99) and is diagrammatically illustrated in Figures 4 and 5. In the normal synthetic process, cholinephosphotransferase catalyzes the interaction of CDP-choline and diacylglycerol to produce phosphatidylcholine and cytidine-5'-monophosphate (Fig. 4, top). During ischemia, the cytidine-5'-monophosphate is not removed because ATP is no longer produced, and its increased concentration reverses the normal reaction, producing more diglycerols that are then rapidly degraded to free fatty acids (Fig. 4, bottom). If the reversal of cholinephosphotransferase and increased fatty acid formation are involved during ischemia, then supplying exogenous citicoline to increase CDP-choline levels would, by mass action principle, reverse this effect and decrease phospholipid degradation, thus favoring recovery from abnormal free fatty acid production (Fig. 5).

Head Trauma. A compound that protects neurons from ischemic damage and might even stimulate cellular growth and repair could have obvious utility in the treatment of head injuries. Studies have been performed to evaluate the effects of citicoline in a number of classical preclinical models designed to approximate one or more parameters identified as significant components in clinical head trauma; actions of citicoline on head trauma reported in eight different models in five species are summarized in Table II.

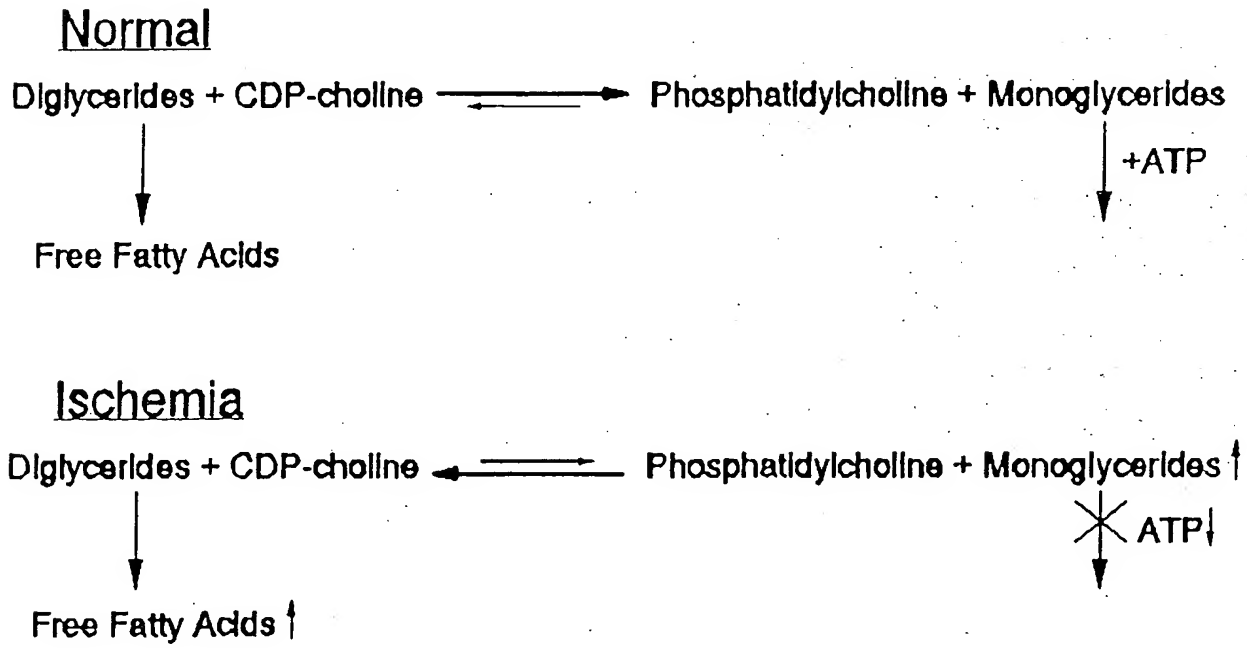

Fig. 4

Diagrammatic illustration of normal synthesis of phosphatidylcholine and of the effect of ischemia on this reaction. Based upon discussion in (99). For explanation, see text.

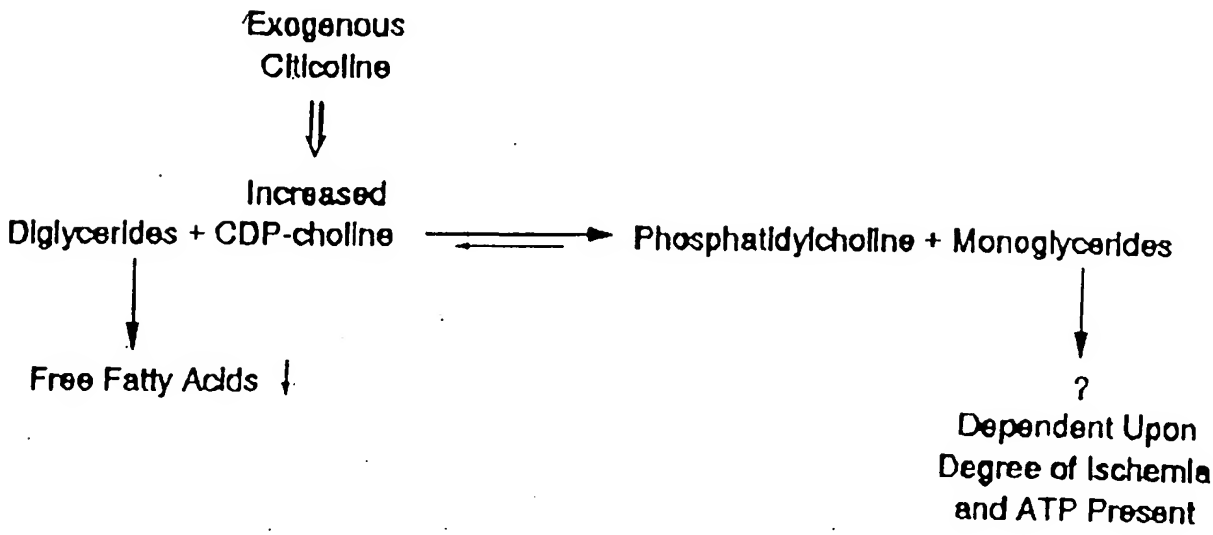

Fig. 5

Diagrammatic illustration of reversal by exogenous citi-coline of increased free fatty acid formation and loss of phosphatidylcholine. Based upon discussion in (99). For explanation, see text.

In a radioautographic study in mice, ^{3}H-labeled citicoline was administered intraperitoneally just after an experimental external head injury (a cold injury) was created by directly applying acetone-dry ice to the scalp of a mouse parietal section for 30 seconds (84). The cerebrum was removed 6 hours later, autoradio-graphs were prepared and the distribution of isotope was compared in normal and injured cells. Incorporation into white matter was much greater in injured cells than in white matter that had sustained no injury.

Effects of citicoline in a concussive head injury model in mice also have been reported (108). Groups of approximately 10 mice at each intravenous dose level of citicoline (0, 1, 2, 4, 8, 15, 30, 60, 125, or 250 mg/kg) were given concussive head injuries 10 minutes after receiving drug, and the interval times until the righting reflex and spontaneous movements reappeared were measured. Significant reductions in time to recovery were observed for both parameters at 60 mg/kg or higher dosages. Uniformity of injury was attempted by dropping a 20 g bakelite weight onto the top of the head of the restrained animal from a height of 30 cm. A more sophisticated version of this model, a controlled cortical impact model of traumatic brain injury based upon devices developed at the General Motors Research Laboratory, has been characterized recently in rats (e.g., 109) and provides additional support for the relevance of this experimental approach to human head injuries. Use of a pneumatic piston rod with controlled velocities permits graded effects to be obtained in approximating many features of severe human traumatic brain injury.

In a cranio-cervical trauma model, rats were subjected to accelerative and decelerative head movements intended to reproduce whiplash with one-half of the traumatized rats (10 rats) receiving citicoline (20 mg/kg, intraperitoneally) 1 hour after the trauma (110). Treatment with citicoline prevented or decreased all of the changes observed (myogram changes; postural regulation, sound avoidance conditioning, changes in catecholamine levels) in untreated traumatized animals. In a rat experimental hematoma model (111), a unilateral cerebral hematoma was prepared by injection of 0.25 mL of autologous blood into the regions regulating motor functions (around capsula interna, putamen and caudate nucleus of the left cerebral hemisphere). The effects of YM-14673, a thyrotro-

TABLE II

Effects of Citicoline in Diverse Preclinical Models of Head Trauma

Model	Species	Effects of Citicoline	Reference(s)
Cold injury lesion	Mouse	Increased incoporation from ^{3}H-Citicoline in injured white (neuronal) matter	(84)
Weight drop concussive head injury	Mouse	Decrease in time to recovery of righting reflex and of spontaneous movement	(108)
Controlled cortical impact model	Rat	Not given; model parameters only	(109)
Cranio-cervical trauma (whip lash)	Rat	Prevented changes in all measured effects (myogram changes, postural regulation, sound avoidance conditioning, dopamine and norepinephrine levels)	(110)
Experimental cerebal hematoma	Rat	Did not affect neurological deficits although YM-14673 (a TRH analog) did	(111)
Cold injury lesion	Rabbit	Decreased edema and restored mitochondrial ATPase and Na^+-K^+-ATPase sensitivity	(112)
Cold injury lesion	Rabbit	Inhibited activation of phospholipase A_2 (but not of cholinephosphotransferase) when given orally	(113)
Experimental cerebral hemorrhage	Dog	EEG parameters improved in 7 of 9 dogs	(114)
UV-induced edema	Cat	Decreased edema	(115, 116)
Epidural brain compression	Cat	Increased resistance to effects (abnormal EEG, cardiovascular and respiratory effects, lethal effects)	(117)

pin-releasing hormone analog, were compared with those of citicoline on alleviation of induced neurological deficits such as hemiplegia. In this model, recovery from neuro-logical deficits was accelerated by YM-14673 but not by citicoline even in concentrations as high as 300 mg/kg. The mechanism of action of citicoline differs from that of thyrotropin-releasing hormones, and citicoline may not act directly to enhance pyramidal motor system activities.

Cold injury in rabbits was induced with liquid nitrogen cryode application (35 sec) through the intact bone in order to induce edema and characterize the underlying mechanisms involved (112). Subcellular fractions were isolated from cerebral hemispheres removed at different time intervals after the cold injury; and cell water and electrolytes, oxidative phosphorylation, and Na^+-K^+-ATPase function were assessed. Citicoline (concentration unspecified) was injected intravenously 24 hours after the cold injury. It acted to stop edema progression, to restore the sensitivity of the mitochondrial ATPase to oligomycin (restoration of the mitochondrial membrane), and to restore Na^+-K^+-ATPase function (membrane phospholipid dependent). In another cold injury study in rabbits (113), citicoline (100-200 mg/kg, orally) completely inhibited injury-mediated activation of phospholipase A_2 (which mediates phospholipid breakdown) but not activation of cholinephosphotransferase activity (which enhances phospholipid biosynthesis). Thus, actions of citicoline in both of these studies could be interpreted as preventing destruction of membrane phospholipids.

Cerebral hemorrhage was surgically induced in nine dogs, and citicoline was subsequently given in intravenous doses of 100-600 mg to ascertain whether it induced improvements in electroencephalographic recordings (114). Although great variability was observed in the condition of the experimental animals and the degree of responsiveness, it was concluded that citicoline was effective in improving EEGs in seven of nine dogs, probably by promoting the arousal reaction.

The effects of citicoline (from 20 to 100 mg/kg) on brain edema was further studied in a cat model of ultraviolet-induced cerebral edema (115, 116). After sacrifice, brain sections were removed and the degree of edema ascertained. Statistically significant reductions of brain edema in relation to the control group were found in the majority of brain levels analyzed in these studies. Further analyses of the mechanisms by which citicoline might decrease brain edema were not performed in these studies but are presumed to relate to restoration of membrane function.

Another experimental technique used in cats was epidural compression of the brain by infusing isotonic saline into a balloon catheter that had been surgically fixed in position in the skull (117). Cats received citicoline (0.833 or 0.167 g/kg TID) for 5 days preceding the surgery and a final dose one hour after surgery and preceding initiation of compression. The results indicate that citicoline moderately protects against the effects of acute brain compression by delaying the onset of abnormal EEG waveforms, large fluctuations in blood pressure and heart rate, respiration changes, cardiac and respiratory failure, and death.

EEG. In several studies in addition to the preceding one (114), effects of citicoline on EEG and/or EMG recordings were measured in attempts to characterize the mode of action of citicoline and whether the changes observed could be used to assess degree of improvement in a preclinical model. In the rat, the cold injury lesion model was used in conjunction with citicoline to assess whether simultaneous administration of citicoline (1 g/kg orally for 8 days) decreased the EEG changes induced by experimental brain edema (118). A significant increase in the theta frequency accompanied by a decrease in the slow alpha and delta frequencies was interpreted as indicating a decrease in the diffuse neuronal damage caused by the cerebral edema. In studies measuring effects

of citicoline on EEG and EMG parameters in rabbits (119-121), it was concluded that changes elicited with citicoline were consistent with an arousal of consciousness, an intensification of pyramidal system activity, an inhibition of myogenic tonus rigidity, and a general facilitation of both sensory and motor systems. Although results were primarily descriptive rather than mechanism-related, comparison with effects of CNS stimulants and other active agents clearly indicates that the spectrum of activity of citicoline was novel.

Dyskinesia. The ability of citicoline and its choline derivative to enhance acetylcholine synthesis could positively affect decreased or aberrant cholinergic function that resulted in hyperactivity. With use of haloperidol to induce hypersensitivity and apomorphine to induce hypermotility, rats receiving citicoline (500 mg/kg/day for 7 days) in this complex model showed hypermotility but a reduction of the apomorphine-induced hypermotility (122). Effects of citicoline observed were attributed to either enhancing actions of increased acetylcholine on the dopaminergic system or an antagonism of the effects of apomorphine (via increased dopamine). Support for the idea that cholinergic changes influence CNS hyperactivity is provided in a study of ^{3}H-choline distribution in rats during and after bicuculline-induced seizures (123). During seizures, there is an increase observed in cerebellar pools of both citicoline and phosphocholine (presumably due to degrading of phosphatidylcholine through the Kennedy pathway).

Parkinson's Disease. Modulation of adrenergic and, especially, dopaminergic CNS systems by synthesis of endogenous acetylcholine and subsequent cholinergic nerve secretion appears to provide the basis for many of the studies on the influence of citicoline in Parkinson's disease (124). The mobilization of the citicoline metabolite, choline, in the stimulation of acetylcholine release and in protecting membrane phospholipids has been documented in rat striatal slices (125). However, as noted in the previous section, orally administered citicoline in the rat haloperidol-apomorphine model appears to have additional actions (e.g., resembling a dopaminergic agonist by activating D_2 receptors; [126]). Another example of complexity is the reported (127) increased formation, by an uncertain and possibly additional mechanism, of CDP-choline in dog brain homogenates induced by stimulation by hemicholinium-3 of the enzyme CTP-phosphocholine cytidylyltransferase.

Considerable experimental evidence of effects of citicoline on CNS dopaminergic systems has accumulated. After treatment with citicoline, regeneration of nerve cells in rats with substantia nigra lesions has been demonstrated (128). In rats, intraperitoneal citicoline administration increased striatal dopamine synthesis as measured by accumulation of the metabolite, DOPA (129). The protective effect of citicoline against decreased caudal dopamine was postulated to result from an improvement in the phospholipid metabolism of injured nerve cells in the substantia nigra and nigrostriatal tract and a subsequent augmentation of dopamine production (130). In rats, citicoline has also been reported to activate tyrosine hydroxylase (131), to increase levels of dopamine and tyrosine in the corpus striatum and to decrease serotonin and tryptophan levels in the hypothalamus and the brain stem (132, 133), to affect synaptosomal transport of various biogenic amines in the corpus striatum (134), to increase the dopamine metabolites, homovanillic acid and DOPAC, in the striatum (135, 136), to increase urinary levels of the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylglycol (137), and to potentiate the circling behavior produced by L-dopa and by amphetamine (133, 138). Thus, although the precise mechanisms by which citicoline directly or indirectly affects CNS biogenic amine function require more definition, activities of citicoline have been demonstrated in preclinical systems relevant to Parkinson's disease, and the specific enhancement of dopaminergic synthetic pathways by citicoline-induced increases in endogenous acetylcholine in the striatum provides a basis for further studies.

Brain Lipids/Aging. The presence of active biosynthesis and repair in neuronal tissues indicates brain structural lipid in membranes is also undergoing turnover under normal conditions. Animal model research has indicated that changes in the composition of dietary fat can alter fatty acid constituents and synthesis of brain membrane lipids (139, 140). Thus, in growing animals, these studies show that the balance of soybean and sunflower oil in the diet influences brain phospholipid fatty acid composition, phosphatidylethanolamine methyltransferase activity, and the rate of phosphatidylcholine biosynthesis via the CDP-choline pathway. Numerous changes have been reported after chronic treatment with citicoline including increased RNA labeling in the corpus striatum of 24-month-old rats (141), reversal of age-dependent modifications of inner mitochondrial membrane and synaptosomal plasma membrane proteins from different rat brain regions (142), incorporation of citicoline during myelination in rat brain organ culture into lecithin and sphingomyelin (143), decreased rat brain mitochondrial citrate synthase (144), a partial recovery of the murine striatum dopamine and acetylcholine receptor function normally reduced with aging (145), and increased incorporation of nucleotide and water-soluble related compounds in various rat brain areas (146).

After chronic administration of citicoline, brain levels of phosphatidylcholine and other structural phospholipids were increased (with ratios essentially unchanged) in mice treated for up to 27 months (147) and in 30-month-old rats as compared with age-related reduced levels in age-matched controls (148). In rat brain cell cultures and slices, repeated exposure to exogenous citicoline increases polyphosphoinositide turnover, and this effect has been attributed to an increased availability of acetylcholine at muscarinic receptors (149). When tissue levels of major membrane phospholipids and their metabolites were measured in three cortical areas from postmortem brains of Alzheimer's disease patients and matched controls (150), there were significant decreases in phospholipids and phospholipid precursors as well as increases in phospholipid catabolites that were consistent with the idea that membrane phospholipid degradation is increased in the Alzheimer's disease brain. The theoretical concept of an initial cholinergic-related lesion in Alzheimer's disease has been proposed (151) as a sequence involving increased mobilization of choline for acetylcholine synthesis from membrane phospholipid (especially phosphatidylcholine) so that membranes are weakened, destroyed, and decreased in quantity. In this scenario, the pathological acceleration of this process in Alzheimer's disease might be mitigated by supplying exogenous acetylcholine precursor, perhaps citicoline. The preclinical basis and relevant therapeutic approaches to Alzheimer-type senile dementia and Alzheimer's disease have been discussed in more detail in recent reviews (152, 153), and no specific studies directly linking citicoline to an effect on parameters of Alzheimer's disease are available.

Learning/Memory. Parallel to situations where aging is accompanied by impaired function are similar experimental models that have been related to deficiencies in memory or learning. In rodent models testing for passive avoidance or conditioned reflex changes, citicoline has been found to restore performance in 13-month-old mice to the level of 4-month-old ones (154) or to improve cognitive levels in rats and mice, but to do so most efficiently in low capability learners (155). Results of citicoline administration to rats in which the degree of induced memory deficits was examined by active and passive avoidance techniques indicate that citicoline may act as a memory-enhancing drug and have a stronger effect in animals with memory deficit. In studies of Petkov and associates, citicoline was reported to prevent scopolamine-induced amnesia (156-159), to facilitate learning and improve memory in rats of all ages with some variability that was not age-dependent (160), and to improve retention when the step-through method for passive avoidance was used (157).

In some situations, however, large variations between and within groups prevented detection of any consistent differences (161). When the effects of the thyrotropin-releasing hormone analog, YM-

14673, were compared with those of citicoline on decreasing latency time for rodents with induced memory deficits to enter from illuminated into dark compartments (passive avoidance), YM-14673 was effective but citicoline was not (162). Memory deficits in rats exposed perinatally to alcohol were decreased by citicoline, but not as well as by piracetam or meclofenoxate (163). The combination of citicoline with the CNS stimulant, caffeine, did not always increase, and occasionally decreased, favorable effects on learning and memory, possibly by disturbing selective pathways (164). It was concluded that antihypoxic effects of various nootropic agents are mediated by actions at differing points affecting the generation of the CDP-choline pool (165). However, the different agents had differing effects on catecholamine and serotonin levels in rat brain areas, with citicoline increasing norepinephrine levels in the cerebral cortex and hypothalamus, dopamine levels in the striatum, and serotonin levels in the cerebral cortex, striatum, and hippocampus (166). Variable effects were reported for citicoline on rat muscarinic receptors (167). Thus, it would appear that the basis of the positive effects of citicoline on memory and learning is complex and difficult to resolve in behavioral models.

Cardiovascular. The effects of citicoline on various cardiovascular and respiratory parameters were measured in anesthetized rats (168). Mean arterial pressure, heart rate, inspiratory flow, expiratory flow, and respiratory rhythm were measured 30 minutes before administration of a single dose of citicoline (1 g/kg, intraduodenally) and up to 2 hours after the dose. No significant differences as compared with controls were observed in heart and respiratory rates. The values for arterial pressure and respiratory flow for citicoline-treated animals remained closer to basal values than did those for control animals. Thus, the measured cardiovascular and respiratory effects of citicoline appear to be minimal at the relatively high dose administered.

A number of other cardiovascular effects and activities of citicoline have been reported. The effects of acute (250 mg/kg) and chronic (250 mg/kg for 2 weeks) treatments with citicoline on platelet aggregation and thromboxane formation and on the platelet antiaggregatory activity of thoracic aorta have been studied in the rat (169). After acute treatment, platelet reactivity to aggregating agents and the antiaggregatory activity of aortic walls were reduced; after chronic treatment, antiaggregatory activity of the aortic walls was increased but platelet function was no longer affected. An antiatherogenic effect of citicoline was also identified in rabbits subjected to 1-2% hypercholesterolemic diets with the diet-induced intimal involvement being reduced by citicoline injections both in the aortic arch and the descending tract of the thoracic aorta (170).

In several publications over a decade, Choy and co-workers (171-173) delineated alterations in the Kennedy pathway in myopathic hamster hearts under normal, hypoxic and ischemic conditions. In myopathic hamster hearts (171), the pool size of CDP-choline was reduced, possibly due to a reduction in cytidine triphosphate precursor (accompanied by a compensatory increase in cytidylyltrans-ferase to maintain a minimum CDP-choline level). In the hypoxic hamster heart (perfused with 95% N_2 saturated buffer), there was a severe decrease in cytidine triphosphate levels as well as a corresponding fall in the conversion of phosphocholine to CDP-choline and the rate of phosphatidylcholine biosynthesis (173). The hypoxia enhanced either lipid activators or translocation of cytidylyltransferase from the cytosolic to the more active microsomal form (mechanisms to compensate for the decreased cytidine triphosphate and to maintain phosphatidylcholine biosynthesis). Finally, in a comparison of ischemic (produced by reduced flow) and hypoxic hearts (172), ischemia produced a severe decrease in ATP levels and consequent decreases in conversion of choline to phosphocholine and reduction (51%) in phosphatidylcholine biosynthesis. In contrast to hypoxia, no endogenous compensatory mechanism was triggered during ischemia. In anoxic cultured rat heart cells (174), treatment with citicoline prevented a significant portion of the decrease in contractility

induced by anoxia. Thus, exogenous citicoline appears to have no serious negative effects on cardiovascular parameters and functions and may be beneficial in the compromised heart.

Induced Lesions. The general protective effects of citicoline on cellular metabolic function were examined in mice given a normally lethal (7.5 mg/kg) dose of potassium cyanide (175). Oral administration of citicoline at a daily dose of 1 g/kg for 4 days significantly extends survival time and increases the percentage of survivors from the effects of KCN-induced histotoxic hypoxia. Some summation with protective effects observed with L-dopa was also shown; but, in a different study in mice (176), ischemia induced by carotid artery ligation decreased dopamine content, and citicoline (0.1 g/kg, intraperitoneally) did not alter this effect.

In several studies, citicoline appears to exert a general protective action against cholinergic stimulants. In mice treated with citicoline before receiving graded intravenous doses of nicotine, the LD_{50} was significantly increased as compared with controls (177). In mice treated with citicoline before receiving oxotremorine (178, 179), citicoline protected against sialorrhea (salivation, lacrimation), diarrhea and hypothermia but not against tremors produced by CNS stimulation. The effects of citicoline were attributed to stimulation of dopamimetic activity (178). In an attempt to modify the mobilization of membrane phospholipids for the synthesis of acetylcholine after stimulated release of neurotransmitter by exposure of electrically polarized corpus striatum slices to the acetylcholinesterase inhibitor, physostigmine (180), exogenous choline was supplied and phospholipid content measured. It was found that addition of choline (or, presumably, a choline source such as citicoline) helps replace acetylcholine without destruction of membrane phospholipid to generate choline. Stimulation with generally cholinergic effects is also observed in the experimental withdrawal syndrome (181) elicited in mice by discontinuing morphine or with naloxone, and the intensity of the physical signs is clearly decreased by administration of citicoline (2 g/kg orally) during the withdrawal period. In rats either with behavioral deficits or receiving drugs that interfere with normal function by differing effects on central cholinergic mechanisms (182), it was found that chronic intraperitoneal injection of citicoline improved memory and learning capacity of all groups.

Effects of citicoline on neuronal regeneration were examined by inducing nerve axon degeneration (183) or by exposure to alcohol (184). Injection of acrylamide, an organic solvent, into mice or rats induces axonal lesions to produce a neurological syndrome for which citicoline is either preventative or hastens recovery (183). When citicoline was administered to pregnant rats also receiving alcohol, effects of alcohol on fetal neuronal development decreased (184).

Conclusions

The potential use of citicoline in a variety of therapeutic indications, especially ischemic and traumatic injuries, is suggested by the extensive and diverse literature that has accumulated especially over the last two decades. The metabolic pathways for CDP-choline have been extensively studied, are well characterized, and are of major physiological significance. The formation of CDP-choline is the rate-limiting step in the synthetic pathway leading from choline to phosphatidylcholine. The pathway is essential for cellular and intracellular membrane formation and maintenance, and CDP-choline is synthesized and mobilized within the intracellular water compartments in virtually all cell types and in all species studied. The hydrolysis products of CDP-choline (cytidine and choline) are primarily incorporated into nucleic acids and phospholipids, respectively. Choline is also essential for synthesis of acetylcholine. Cytidine triphosphate formed from cytidine increases choline incorporation into membrane phosphatidylcholine.

The oral administration of citicoline has been shown to augment various physiologically beneficial actions of endogenous CDP-choline. Exogenously administered citicoline is orally bioavailable. After hydrolysis in the intestine to choline and cytidine, these constituents are absorbed, widely distributed, resynthesized in liver and other cells, and efficiently used in brain cells for membrane phosphatidylcholine synthesis. The effects of ischemia and/or hypoxia in most animal and cellular model systems studied are prevented, reduced, or reversed by exogenously administered citicoline. Citicoline not only increases phospholipid synthesis but also inhibits phospholipid degradation. In head trauma models, citicoline acts to decrease and limit nerve cell membrane damage and, in this manner, restores intracellular regulatory enzyme sensitivity and function, and limits edema. Beneficial activity of exogenous citicoline has also been postulated or reported for dyskinesia, Parkinson's disease, cardiovascular disease, aging, Alzheimer's disease, learning and memory, and cholinergic stimulation. Citicoline reverses or prevents cell membrane damage and subsequent deterioration in many animal and cellular models without serious side effects.

References

1. E.P. KENNEDY, Harvey Lect. 57 143-171 (1962).
2. E.P. KENNEDY and S.B. WEISS, J Biol Chem. 222 193-214 (1956).
3. D.E. VANCE, Biochem Cell Biol. 68 1151-1165(1990).
4. M.C. SANCHEZ, J.M. FERNANDEZ, E. FORNE, J. CASTELLO, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1011-1012 (1983).
5. R. ROBERTI, A. MANCINI, L. FREYSZ and L. BINAGLIA, Biochim Biophys Acta. 1165 183-188 (1992).
6. M.L. THOM and R.D. ZACHMAN, Pediatr Res. 9 201-205 (1975).
7. A. FLORIDI, A. VECCHINI, C.A. PALMERINI, L. BINAGLIA and G. PORCELLATI, Meeting of the Italian Biochemical Society. 30 317-319 (1981).
8. I. LOPEZ G-COVIELLA and R.J. WURTMAN, J Neurochem. 59 338-343 (1992).
9. D.S. HAINES and S.D. TOKMAKJIAN, Biochem Cell Biol. 65 105-111 (1987).
10. G. ARIENTI, L. CORAZZI, P. MASTROFINI, I. MONTANINI, B. TIRILLINI, and G. PORCELLATI, Ital J Biochem. 28 39-45 (1979).
11. R. SLEIGHT and C. KENT, J Biol Chem. 255 10644-10650 (1980).
12. P.S. SUNGA, P.H. PRITCHARD, and S.W. RABKIN, Biochem Cell Biol. 66 231-237 (1988).
13. P.H. PRITCHARD, P.K. CHIANG, G.L. CANTONI, and D.E. VANCE, J Biol Chem. 257 6362-6367 (1982).
14. P.H. PRITCHARD and D.E. VANCE, Biochem J. 196 261-267 (1981).
15. R.B. CORNELL and H. GOLDFINE, Biochim Biophys Acta. 750 504-520 (1983).
16. M. POST, J.J. BATENBURG, E.A. SCHUURMANS and L.M. VAN GOLDE, Biochim Biophys Acta. 712 390-394 (1982).
17. T.A. ZELINSKI, J.D. SAVARD, R.Y. MAN and P.E. CHOY, J Biol Chem. 255 11423-11428 (1980).
18. M. WIENTZEK, R.Y. MAN and P.C. CHOY, Biochem Cell Biol. 65 860-868 (1987).
19. J.P. INFANTE, FEBS Lett. 214 149-152 (1987).
20. J.M. MATO and S.ALEMANY, Biochem J. 213 1-10 (1983).
21. G.M. HATCH, K.O, P.C.CHOY Biochem Cell Biol. 67 67-77 (1989).
22. A. VECCHINI, L. BINAGLIA, C.A. PALMERINI, A. FLORIDI and G. PORCELLATI, Ital J Biochem. 30 367-374 (1981).
23. G.P. VAN HEUSDEN and H. VAN DEN BOSCH, Biochim Biophys Acta. 711 361-368 (1982).
24. B.E. MILLER and G.E. HOOK, Am J Respir Cell Mol Biol. 1 127-136 (1989).
25. H.K. STALS, G.P. MANNAERTS and P.E. DECLERCQ, Biochem J. 283 719-725 (1992).
26. J.E. VANCE and D.E. VANCE, Can J Biochem Cell Biol. 63 870-881 (1985).
27. F.G. TOBACK, L.J. HAVENER, R.D. DODD and B.H. SPARGO, Am J Physiol. 232 E216-E222 (1977).
28. F.G. TOBACK, L.J. HAVENER and B.H. SPARGO, Am J Physiol. 233 E212-E218 (1977).
29. S. TOKMAKJIAN and F. POSSMAYER, Biochim Biophys Acta. 666 176-180 (1981).
30. S. TOKMAKJIAN, D.S. HAINES and F. POSSMAYER, Biochim Biophys Acta. 663 557-568 (1981).

31. G. ARIENTI, L. CORAZZI, H. WOELK and G. PORCELLATI, J Neurochem. 27 203-210 (1976).
32. G. TROVARELLI, C.A. PALMERINI, A. FLORIDI, G.L. PICCININ and G. PORCELLATI, Neurochem Res. 9 73-79 (1984).
33. G. TROVARELLI, C.A. PALMERINI, A. FLORIDI, G.L. PICCININ and G. PORCELLATI, Neurochem Res. 7 1199-1207 (1982).
34. R. SUNDLER and B. AKESSON, Biochem J. 146 309-315 (1975).
35. F.A. SHAMGAR and F.D. COLLINS, Biochem Biophys Res Commun. 76 550-558 (1977).
36. R.D. ZACHMAN, P.W. COTTER and F.H. TSAO, Biol Neonate. 44 129-137 (1983).
37. E. FRANCESCANGELI, G. GORACCI, G.L. PICCININ, R. MOZZI, H. WOELK and G. PORCELLATI, J Neurochem. 28 171-176 (1977).
38. G.S. COBON, P.D. CROWFOOT and A.W. LINNANE, Biochem J. 144 265-275 (1974).
39. T.S. REDDY and L.A. HORROCKS, Int J Dev Neurosci. 4 89-95 (1986).
40. L. FREYSZ, L. BINAGLIA, H. DREYFUS, R. MASSARELLI, F. GOLLY and G. PORCELLATI J Neurochem. 45 57-62 (1985).
41. J.A. HIGGINS, Biochim Biophys Acta. 558 48-57 (1979).
42. D.E. VANCE, P.C. CHOY, S.B. FARREN, P.H. LIM and W.J. SCHNEIDER, Nature. 270 268-269 (1977).
43. K.M. HARGREAVES and M.T. CLANDININ, Biochim Biophys Acta. 1001 262-267 (1989).
44. K.M. HARGREAVES and M.T. CLANDININ, Biochem Biophys Res Commun. 145 309-315 (1987).
45. J.E. VANCE, D. PAN, D.E. VANCE and R.B. CAMPENOT, J Cell Biol. 115 1061-1068 (1991).
46. J.E. VANCE and D.E. VANCE, J Biol Chem. 261 4486-4491 (1986).
47. R. RUSTOW and D. KUNZE, Biochim Biophys Acta. 835 273-278 (1985).
48. L. BINAGLIA, R. ROBERTI, A. VECCHINI and G. PORCELLATI, J Lipid Res. 23 955-961 (1982).
49. T.P. GEORGE, S.C. MORASH, H.W. COOK, D.M. BYERS, F.B. PALMER and M.W. SPENCE, Biochim Biophys Acta. 1004 283-291 (1989).
50. T.P. GEORGE, H.W. COOK, D.M. BYERS, F.B. PALMER and M.W. SPENCE, J Biol Chem. 266 12419-12423 (1991).
51. S. ALEMANY, I. VARELA and J.M. MATO, Biochem J. 208 453-457 (1982).
52. L.J. HAVENER and F.G. TOBACK, J Clin Invest. 65 741-745 (1980).
53. G.M. HATCH and P.C. CHOY, Lipids. 22 672-676 (1987).
54. I.H. ULUS, R.J. WURTMAN, C. MAURON and J.K. BLUSZTAJN, Brain Res. 484 217-227 (1989).
55. K. SHIMAMOTO and Y. ARAMAKI, J Takeda Res Lab. 34 189-201 (1975).
56. P. GALLETTI, M. DE ROSA, M.A. NAPPI, G. PONTONI, L. DEL PIANO, A. SALUZZO and V. ZAPPIA, Biochem Pharmacol. 34 4121-4130 (1985).
57. E. FRANCESCANGELI and G. GORACCI, Biochem Biophys Res Commun. 161 107-112 (1989).
58. T.C. LEE, B. MALONE, M.L. BLANK, V. FITZGERALD and F. SNYDER, J Biol Chem. 265 9181-9187 (1990).
59. D.S. WOODARD, T.C. LEE and F. SNYDER, J Biol Chem. 262 2520-2527 (1987).
60. R.F. VILLA, D. CURTI, M. POLGATTI and G. BENZI, J Neurosci Res. 7 341-347 (1982).
61. R.F. VILLA, F. MARZATICO and G. BENZI, Neurochem Res. 8 269-290 (1983).
62. R.N. KOLESNICK, J Biol Chem. 262 14525-14530 (1987).
63. J.M. HOFFMAN and S.G. LAYCHOCK, Diabetes. 37 1489-1498 (1988).
64. R.G. LAMB, J.C. KOCH and S.R. BUSH, Biochim Biophys Acta. 1165 299-305 (1993).
65. H.P. HAAGSMAN, J.M. VAN DEN HEUVEL, L.M. VAN GOLDE and M.J. GEELEN, J Biol Chem. 259 11273-11278 (1984).
66. G. ROMEO, G. COCCHIARA, M. DEROSA, F. GIORDANO and V. ZAPPIA, Boll Soc Ital Biol Sper. 61 1175-1181 (1981).
67. K. YASHIMA, M. TAKAMATSU and K. OKUDA, J Nutr Sci Vitaminol (Tokyo). 21 49-60 (1975).
68. J. AGUT, E. FONT, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1045-1047 (1983).
69. R. PARONI, G. CIGHETTI, M. DEL PUPPO and M.G. KIENLE, Pharmacol Res Commun. 17 805-829 (1985).
70. P. GALLETTI, M. DE ROSA, M.G. COTTICELLI, A. MORANA, R. VACCARO and V. ZAPPIA, J Neurol Sci. 103(Suppl):S19-S25 (1991).

71. I. LOPEZ G-COVIELLA, J. AGUT, R. VON BORSTEL and R.J. WURTMAN, Neurochem Int. 2 293-297 (1987).
72. M. DE ROSA, P. GALLETTI, G. ROMEO, A. NAPPI, G. PONTONI, E. ARRIGONI and V. ZAPPIA, *Novel Biochemical, Pharmacological and Clinical Aspects of Cytidine-diphosphocholine*, V. Zappia, E.P. Kennedy, B.I. Nilsson and G. Galletti (Eds), 139-157, Elsevier, New York (1985).
73. A. SALUZZO, L. DELPIANO and M. DE ROSA, P. GALLETTI, M.A. NAPI, G.L. RUSSO and V. ZAPPIA, Abstract from Summaries and Minutes of the Academy of Medical and Surgical Sciences, Institute of Biochem. of Macromolecules, Univ. of Naples, 140:1-11 (1986).
74. G. ROMEO, G. COCCHIARA, M. DEROSA, A. ANDREANA, G. RUGGIERO and V. ZAPPIA, Boll Soc Ital Biol Sper. 61 1182-1187 (1980).
75. L. KRAWIEC, J.M. PASQUINI, R. NAJLE and E.F. SOTO, Acta Physiol Lat Am. 25 67-76 (1975).
76. J. AGUT, E. FONT, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1048-1050 (1983).
77. M. OGASHIWA, K. TAKEUCHI, M. HARA, Y. TANAKA and J. OKADA, Brain and Nerve. 26 1181-1187 (1974).
78. M. OGASHIWA, K. TAKEUCHI, M. HARA, Y. TANAKA and J. OKADA, Int J Clin Pharmacol. 12 327-335 (1975).
79. A. ROMERO, J. SERRATOSA, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1054-1056 (1983).
80. A. ROMERO, J. SERRATOSA, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1056-1058 (1983).
81. A. ROMERO, J. SERRATOSA, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1058-1060 (1983).
82. J. AGUILAR, R. GIMENEZ, O. BACHS, C. ENRICH and J. AGUT, Arzneim.-Forsch./Drug Res. 33 1051-1053 (1983).
83. A. VECCHINI, L. BINAGLIA, A. FLORIDI, C.A. PALMERINI and G. PORCELLATI, Neurochem Res. 8 333-340 (1983).
84. T. TSUCHIDA, M. NAGAI, T. HOSHINO, S. KAMANO and H. MIYAKE, Brain and Nerve. 19 1041-1045 (1967).
85. R.V. DORMAN, Z. DABROWIECKI and L.A. HORROCKS, J Neurochem. 40 276-279 (1983).
86. W.J. GOLDBERG, R.V. DORMAN, Z. DABROWIECKI and L.A. HORROCKS, Neurochem Pathol. 3 237-248 (1985).
87. L.A. HORROCKS, R.V. DORMAN, Z. DABROWIECKI, G. GORACCI and G. PORCELLATI, Prog Lipid Res. 20 531-534 (1981).
88. L.A. HORROCKS, R.V. DORMAN and G. PORCELLATI, *Cerebral Ischemia*, A. Bos, P. Braquet, R. Paoletti and B.K. Siesis(Eds), 211-222, Elsevier B.V., Amsterdam (1984).
89. M. KAKIHANA, N. FUKUDA, M. SUNO and A. NAGAOKA, Stroke. 19 217-222 (1988).
90. H. SATO, M. HARIYAMA and K. MORIGUCHI, Biochem Biophys Res Commun. 150 491-496 (1988).
91. M. YAMAMOTO, M. SHIMIZU and H. OKAMIYA, Eur J Pharmacol. 181 207-214 (1990).
92. F. BOISMARE, M. LE PONCIN LAFITTE and J.R. RAPIN, Comptes rendus des seances de la Societe' de Biologie. 172 651 (1978).
93. G. SALIGAUT and F. BOISMARE, Med Clin (Barc). 87(Suppl 1) 19-22 (1986).
94. G. SALIGAUT, M. DAOUST, N. MOORE and F. BOISMARE, Arch Int Pharmacodyn Ther. 285 25-33 (1987).
95. G. SALIGAUT, M. DAOUST, N. MOORE, P. CHRETIEN and F. BOISMARE, Circulation et metabolisme du cerveau. 2 33-42 (1984).
96. G. HAMDORF and J. CERVOS-NAVARRO, Arzneim.-Forsch./Drug Res. 40 519-522 (1990).
97. G. HAMDORF and J. CERVOS-NAVARRO, Arzneim.-Forsch./Drug Res. 41 1206-1210 (1991).
98. G. HAMDORF, J. CERVOS-NAVARRO and R. MULLER, Arzneim.-Forsch./Drug Res. 42 421-424 (1992).
99. G.E. DE MEDIO, M. BRUNETTI, R.V. DORMAN, B. DROZ, L.A. HORROCKS, G. PORCELLATI, F. SOUYRI and G. TROVARELLI, Birth Defects: Original Article Series. 19 175-187 (1983).
100. G. TROVARELLI, G.E. DE MEDIO and I. MONTANINI, Farmaco (Sci). 37 663-668 (1982).

101. G. TROVARELLI, G.E. DEMEDIO, R.V. DORMAN, G.L. PICININ, L.A. HORROCKS and G. PORCELLATI, Neurochem Res. 6 821-833 (1981).
102. M. ALBERGHINA, M. VIOLA, I. SERRA, A. MISTRETTA and A.M. GIUFFRIDA, J Neurosci Res. 6 421-433 (1981).
103. I. SERRA, M. ALBERGHINA, M. VIOLA, A. MISTRETTA and A.M. GIUFFRIDA, Neurochem Res. 6 607-618 (1981).
104. H. NAGANO, T. SUZUKI, M. HAYASHI and M. ASANO, In Vivo. 6 497-502 (1992).
105. G. BENZI, E. ARRIGONI, O. PASTORIS, R.F. VILLA, M. DOSSENA, A. AGNOLI and A. M. GIUFFRIDA, J Cereb Blood Flow Metab. 2 229-239 (1982).
106. S. MYKITA, F. GOLLY, H. DREYFUS, L. FREYSZ and R. MASSARELLI, J Neurochem. 47 223-231 (1986).
107. M. ALBERGHINA, M. VIOLA and A.M. GIUFFRIDA, J Neurosci Res. 6 719-722 (1981).
108. M. OGASHIWA, K. SANO, S. MANAKA, K. KITAMURA, M. KAGAWA and K. TAKEUCHI, *Novel Biochemical, Pharmacological and Clinical Aspects of Cytidinediphosphocholine*, V. Zappia, E. Kennedy, B.I. Nilsson and P. Galletti(Eds), 317-327, Elsevier, New York (1985).
109. C.E. DIXON, G.L. CLIFTON, J.W. LIGHTHALL, A.A. YAGHMAI and R.L. HAYES, J Neurosci Methods. 1-10 (1991).
110. F. BOISMARE, M. LE PONCIN, J. LEFRANCOIS, L. HACPILLE and J.C. MARCHAND, Therapie. 32 345-354 (1977).
111. M. YAMAMOTO, M. SHIMIZU, S. KAWABATA and M. TERAI, Arch Int Pharmacodyn Ther. 299 55-64 (1989).
112. M. RIGOULET, B. GUERIN, F. COHADON and M. VANDENDREISSCHE, J Neurochem. 32 535-541 (1979).
113. E. ARRIGONI, N. AVERET and F. COHADON, Biochem Pharmacol. 36 3697-3700 (1987).
114. M. KASE, J. ONO, N. YOSHIMASU, E. HIYAMUTA, K. SUGIURA, R. ITO and K. HAMASAKI, Curr Ther Res. 16 483-502 (1974).
115. J. CERVOS NAVARRO and J.V. LAFUENTE, Adv Neurol. 52 421-429 (1990).
116. J.V. LAFUENTE SANCHEZ and J. CERVOS NAVARRO, Med Clin (Barc). 87 (Suppl 1):5-8 (1986).
117. D.R. ALGATE, D.J. BEARD, A. SACRISTAN, J.A. ORTIZ and J.E. DAVIES, Arzneim.-Forsch./Drug Res. 33 1013-1016 (1983).
118. X. MAJEM, A. BIDON CHANAL and J. VILA BADO, Med Clin (Barc). 87 (Suppl 1):23-25 (1986).
119. T. MATSUNAGA and S.I. OKUMURA, Med J Osaka Univ. 31 55-62 (1980).
120. M. YASUHARA and H. NAITO, Curr Ther Res. 16 346-374 (1974).
121. M. YASUHARA and H. NAITO, Int J Neurosci. 11 181-194 (1980).
122. J. AGUT, E. FONT, A. SACRISTAN and J.A. ORTIZ, Med Clin (Barc). 87 (Suppl 1):14-18 (1986).
123. L. CORAZZI, G.L. PICCININ, N. MARKU and G. ARIENTI, Neurochem Res. 11 401-406 (1986).
124. R.J. WURTMAN and I. LOPEZ G-COVIELLA, Med Clin (Barc). 87 (Suppl):3-4 (1986).
125. R. LEVENT BUYUKUYSAL, T.C. HOLMES and R.J. WURTMAN, Brain Res. 541 1-6 (1991).
126. J. AGUT, E. FONT, J.M. SALADRICH, A. SACRISTAN and J.A. ORTIZ, Med Clin (Barc). 87 (Suppl 1):11-13 (1986).
127. M.V. GOMEZ, O.Z. SELLINGER, J.C. SANTIAGO and E.F. DOMINO, Int J Neurosci. 2 145-149 (1971).
128. S. STANZANI, Boll Soc Ital Biol Sper. 56 1830-1834 (1980).
129. M.L. PORCEDDU and A. CONCAS, Farmaco (Sci). 40 617-622 (1985).
130. S. MANAKA, K. SANO, T. FUCHINOUE and H. SEKINO, Experientia. 30 179-180 (1974).
131. M. MARTINET, P. FONLUPT and H. PACHECO, Biochem Pharmacol. 30 539-541 (1981).
132. M. MARTINET, P. FONLUPT and H. PACHECO, Arch Int Pharmacodyn. 239 52-61 (1979).
133. M. SHIBUYA, N. KAGEYAMA, T. TANIGUCHI, H. HIDAKA and M. FUJIWARA, Jpn J Pharmacol. 31 47-52 (1981).
134. M. MARTINET, P. FONLUPT and H. PACHECO, Experientia. 34 1197-1199 (1978).
135. J. AGUT, I. LOPEZ G-COVIELLA and R.J. WURTMAN, Neuropharmacology. 23 1403-1406 (1984).
136. J. AGUT, E. FONT, A. SACRISTAN and J.A. ORTIZ, Med Clin (Barc). 87 (Suppl 1):9-10 (1986).
137. I. LOPEZ G-COVIELLA, J. AGUT and R.J. WURTMAN, J Neural Transm. 66 129-134 (1986).

138. C. SALIGAUT, M. DAOUST, N. MOORE and F. BOISMARE, Neuropharmacology. 26 1315-1319 (1987).
139. M.T. CLANDININ, M. SUH and K. HARGREAVES, *Neurobiology of Essential Fatty Acids*, N.G. Bazam et.al. (Eds), 297-308, Plenum Press, New York (1992).
140. K. HARGREAVES and M.T. CLANDININ, Ups J Med Sci Suppl. 48 79-95 (1990).
141. D.F. CONDORELLI, R. AVOLA, N. RAGUSA, S. REALE, M. RENIS, R.F. VILLA and A.M. GIUFFRIDA-STELLA, Neurochem Res. 14 701-706 (1989).
142. R.F. VILLA, F. INGRAO, G. MAGRI, A. GORINI, S. REALE, A. COSTA, M. RAGUSA, R. AVOLA and A.M. GIUFFRIDA-STELLA, Int J Dev Neurosci. 11 83-93 (1993).
143. N. CHIDA and Y. SHIMIZU, Tohoku J Exp Med. 111 41-49 (1973).
144. G. BENZI, E. ARRIGONI, F. DAGANI, F. MARZATICO, D. CURTI, S. RAIMONDO, M. DOSSENA, M. POLGATTI and R.F. VILLA, Exp Gerontol. 15 593-603 (1980).
145. R. GIMENEZ, J. RAICH and J. AGUILAR, Br J Pharmacol. 104 575-578 (1991).
146. G.E. DE MEDIO, G. TROVARELLI, G.L. PICCININ and G. PORCELLATI, J Neurosci Res. 11 49-58 (1984).
147. I. LOPEZ G-COVIELLA, J. AGUT, A. ORTIZ and R.J. WURTMAN, J Nutr Biochem. 3 120-122 (1992).
148. J. AGUT, *Marcadores Biologicosy Perspectivas Terapeuticas de la Entermedal de Alzheimer*, N. Acorun Tusell and J. Alom(Eds), 77-88, Editorial MCR, Barcelona (1989).
149. P.L. CANONICO, F. NICOLETTI, M.A. SORTINO and U. SCAPAGNINI, Funct Neurol. 7 275-281 (1992).
150. R.M. NITSCH, J.K. BLUSZTAJN, A.G. PITTAS, B.E. SLACK, J.H. GROWDON, and R.J. WURTMAN, Proc Natl Acad Sci USA. 89 1671-1675 (1992).
151. R.J. WURTMAN, J.K. BLUSZTAJN, I. LOPEZ G-COVIELLA, M. LOGUE and J.H. GROWDON, *Demencias. Inicio de Una Decada, Los Amos del Progreso?* F. Bermejo and N. Acarin(Eds), 127-134, Editorial MCR, Barcelona (1991).
152. J. JIMENEZ HERRERO, I. FERRER and R. LOZANO, Rev Esp Geriatr Gerontol. 26 (Suppl 2):9-38 (1991).
153. J.M. MARTINEZ-LAGE, Rev Esp Geriatr Gerontol. 24 (Suppl 1):46-61 (1989).
154. J. AGUT and J.A. ORTIZ, Ann NY Acad Sci. 640 295-297 (1991).
155. V.D. PETKOV, A.H. MOSHARROF, R. KEHAYOV, V.V. PETKOV, E. KONSTANTINOVA and D. GETOVA, Methods Find Exp Clin Pharmacol. 14 593-605 (1992).
156. A.H. MOSHARROF and V.D. PETKOV, Acta Physiol Pharmacol Bulg. 16 25-31 (1990).
157. A.H. MOSHARROF, V.D. PETKOV and V.V. PETKOV, Acta Physiol Pharmacol Bulg. 13 17-24 (1987).
158. V.D. PETKOV and A.H. MOSHARROF, Arzneim.-Forsch./Drug Res. 39 1133-1136 (1989).
159. V.D. PETKOV, A.H. MOSHARROF and V.V. PETKOV, Acta Physiol Pharmacol Bulg. 14 3-13 (1988).
160. V.D. PETKOV, A.H. MOSHARROF, V.V. PETKOV and R.A. KEHAYOV, Acta Physiol Pharmacol Bulg. 16 28-36 (1990).
161. V.D. PETKOV and R. KEHAYOV, Acta Physiol Pharmacol Bulg. 13 30-36 (1987).
162. M. YAMAMOTO and M. SHIMIZU, Naunyn Schmiedebergs Arch Pharmacol. 338 262-267 (1988).
163. V.D. PETKOV, E.R. KONSTANTINOVA, V.V. PETKOV and J V. VAGLENOVA, Methods Find Exp Clin Pharmacol. 13 43-50 (1991).
164. V.D. PETKOV, E. KONSTANTINOVA, V.V. PETKOV, M. LAZAROVA and B. PETKOVA, Acta Physiol Pharmacol Bulg. 17 17-26 (1991).
165. H.D. FISCHER, J. SCHMIDT and C. WUSTMANN, Biomed Biochim Acta. 43 541-543 (1984).
166. V.D. PETKOV, S.L. STANCHEVA, L. TOCUSCHIEVA and V.V. PETKOV, Gen Pharmacol. 21 71-75 (1990).
167. V.D. PETKOV and J.S. POPOVA, Acta Physiol Pharmacol Bulg. 13 3-10 (1987).
168. M.A. BRASO, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1043-1045 (1983).
169. I. MASI, E. GIANI and C. GALLI, Pharmacol Res Commun. 18 273-281 (1986).
170. G. WEBER, A. AUTERI, G. BIANCIARDI, P. FABRINI, L. RESI, M. SALVI, P. TOTI and P. TANGANELLI, Drugs Exp Clin Res. 15 321-323 (1989).

171. P.C. CHOY, J Biol Chem. 257 10928-10933 (1982).
172. P.C. CHOY, M. CHAN, G. HATCH and R.Y. MAN, Mol Cell Biochem. 116 53-58 (1992).
173. G.M. HATCH and P.E. CHOY, Biochem J. 268 47-54 (1990).
174. A. NEIDHARDT, Y. COSTES, K. BACHOUR and N. PLATONOFF, Clin Ther. 14 537-543 (1992).
175. M.E. TORNOS, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1022-1024 (1983).
176. H. NAKAMUTA, K. MUGURUMA, M. KOIDA, Y. HIRAMATSU and Y. OGAWA, J Pharmacobiodyn. 12 429-436 (1989).
177. T. GRAU, A. ROMERO, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1025-1026 (1983).
178. C. SALIGAUT, M. DAOUST, M. CHADELAUD, N. MOORE, P. CHRETIEN and F. BOISMARE, Methods Find Exp Clin Pharmacol. 7 5-8 (1985).
179. M.E. TORNOS, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1026-1029 (1983).
180. R.J. WURTMAN, J.K. BLUSZTAJN, J.H. GROWDON and I.H. ULUS, *Current Research in Alzheimer Therapy*, Giacobin and Becker(Eds), 95-100, Taylor & Francis, New York (1988).
181. M.E. TORNOS, A. SACRISTANA and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1018-1021 (1983).
182. F. DRAGO, F. MAUCERI, L. NARDO, C. VALERIO, A.A. GENAZZANI and M. GRASSI, Brain Research Bulletin. 31 485-489 (1993).
183. J. AGUT, E. FONT, A. SACRISTAN and J.A. ORTIZ, Arzneim.-Forsch./Drug Res. 33 1029-1033 (1983).
184. S. PATT, J. CERVOS NAVARRO, G. STOLTENBURG DIDINGER and C. SCHREINER, Histol Histopathol. 4 429-434 (1989).